

APPLICATION OF GC/TOFMS FOR COMPREHENSIVE ANALYSIS OF ORGANOHALOGEN COMPOUNDS IN BLUBBER AND LIVER OF FINLESS PORPOISE

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Abstract

Comprehensive analytical methodology for the investigation of organohalogen compounds in biological sample was developed by gas chromatography/time-of-flight mass spectrometry (GC/TOFMS). Accurate mass measurement with wide scan range on TOFMS helped to pick up organohalogen compounds from complicated matrix components derived from sample. In blubber and liver of finless porpoise, polychlorinated biphenyls (PCBs) were detected at the highest levels, and those of other organohalogen compounds were in the order of dichlorodiphenyltrichloroethane and its metabolites (DDTs) > chlordane related compounds (CHLs) > hexachlorocyclohexane isomers (HCHs) > hexachlorobenzene (HCB) > polychlorinated diphenyl ethers (PCDEs) > hexabromocyclododecane (HBCD) > polybrominated diphenyl ethers (PBDEs).

Introduction

Organohalogen compounds such as polychlorinated dibenzo-*p*-dioxin (PCDDs), polychlorinated dibenzofurans (PCDFs), PCBs, organochlorine pesticides, and brominated flame retardants (BFRs) including PBDEs and HBCD are persistent, toxic and lipophilic chemicals, and these have been shown to bioaccumulate in organisms^{1,2}. Some of PCBs and PBDEs, taken in vivo are metabolized and transformed to hydroxylated and methoxylated derivatives². Additionally, bioaccumulative organohalogen compounds are proposed to be of natural origin. Considering these facts, it is supposed that various pieces of organohalogen compounds are contained in organisms, which are anthropogenic organohalogen compounds (AOCs) such as PCDDs, PCDFs, PCBs, and BFRs, organohalogen compounds transformed from AOCs, and halogenated natural products (HNPs). Comprehensive analytical technique for various organohalogen compounds to confirm the contamination status in organisms is required.

In our previous study^{3,4}, we investigated the comprehensive analytical method for organobromine compounds by GC/double focusing mass spectrometry. Firstly, GC/low resolution mass spectrometry (LRMS, $M/\Delta M > 1,000$) with negative ion chemical ionization (NCI) scanning was performed. In NCI, organohalogen compounds were ionized and showed the specific spectrum of halogen, therefore, mass chromatogram of bromine ion ($m/z = 79, 81$) was useful to assign peaks of organobromine compounds. Secondly, GC/LRMS with electron impact (EI) scanning was performed for identification of organobromine compounds by searching of the mass spectrum in the assigned peaks. GC condition in EI scanning was the same as in NCI. Thirdly, GC/high resolution mass spectrometry (HRMS, $M/\Delta M > 10,000$) with EI scanning was performed accurate mass measurement for determination of molecular formula in unknown compounds. These GC/MS techniques were applied for blubber of finless porpoise, and identified tetra-, penta-, hexa-, and hepta-BDE, methoxylated tetra-BDE (OMe-TeBDE), and tribromo- and tetrabromo- hexahydro-1*H*-xanthene derivatives that are one of the HNPs which reported widespread in nature⁵.

In the present study, we applied GC/TOFMS with EI and NCI to comprehensive analysis of organochlorine and organobromine compounds, and evaluated this method by using blubber and liver of finless porpoise that differed from individual had used in previous study. TOFMS offered the accurate mass spectra in wide scan range with high full scan sensitivity and rapid spectral acquisition rates. The accurate mass obtained from TOFMS helped to determine the elemental compositions of analyte, to distinguish between nominally isobaric components, to distinguish between analyte and matrix related material, to improve database search specificity, and to success in analyte identification, therefore, TOFMS was ideal for full characterization and profiling of components in a whole range of sample types.

Materials and Methods

Blubber (3g) and liver (10g) of finless porpoise were individually homogenized and dehydrated with anhydrous sodium sulfate, soxhlet-extracted with dichloromethane for 16 hours, and crude extracts were concentrated to 20 mL using rotary evaporator. 1 mL of the extracts were purified by sulfuric acid-silicagel column chromatography, concentrated to 0.1 mL and measured by GC/TOFMS.

The procedure of comprehensive analytical method by GC/TOFMS was followed. Firstly, GC/TOFMS with NCI measurement was performed for assignment of peaks of organochlorine and organobromine compounds. Secondly, GC/TOFMS with EI measurement was performed for the determination of the elemental compositions of these compounds that are assigned in NCI. GC condition for EI measurement was the same as NCI, and grasped the approximate levels of these contaminations with accurate mass chromatograms.

GC/TOFMS measurement was performed on a GCT Premier (Waters/Micromass), which offered the high-resolution accurate mass spectra with full scan sensitivity and rapid spectral acquisition rates. We selected the GC column that its length was short (15 m), its film thickness was thin (0.1 μm), and its phase was slightly polar ((5%-phenyl)-methylsiloxane) due to avoid thermal degradation and adsorption of organobromine compounds in GC column with on-column injection. GC column: DB-5MS (15 m x 0.25 mm, 0.1 μm , J&W), GC column temperature: 120°C (1 min)-(10°C/min)-300°C (15 min), carrier gas: He (1.0 mL/min) injector temperature: 120°C-(100°C/min)-300°C, injection volume: 2.0 μL , interface temperature: 300°C, source temperature: 300°C, and using amber glassware and UV cut off light to avoid photo degradation.

Results and Discussion

Figure 1 shows the total ion chromatogram (TIC) and mass chromatograms of bromine ion obtained by GC/TOFMS with NCI measurement and TIC obtained by GC/TOFMS with EI measurement in blubber of finless porpoise. Many organochlorine compounds and several organobromine compounds were assigned by using TIC and mass chromatogram of bromine ion in NCI picked up from complicated matrix components derived from sample. These organohalogen peaks confirmed in NCI were identified from accurate mass spectra in EI.

Figure 2 shows the mass chromatograms of PCBs, organochlorine pesticides, and organobromine compounds by GC/TOFMS with EI measurement in blubber of finless porpoise. Some of the peaks of PCBs and organochlorine pesticides were saturated in this condition. Accordingly, a part of this sample was diluted 100 times with decane, and retried to measurement for PCBs and organochlorine pesticides.

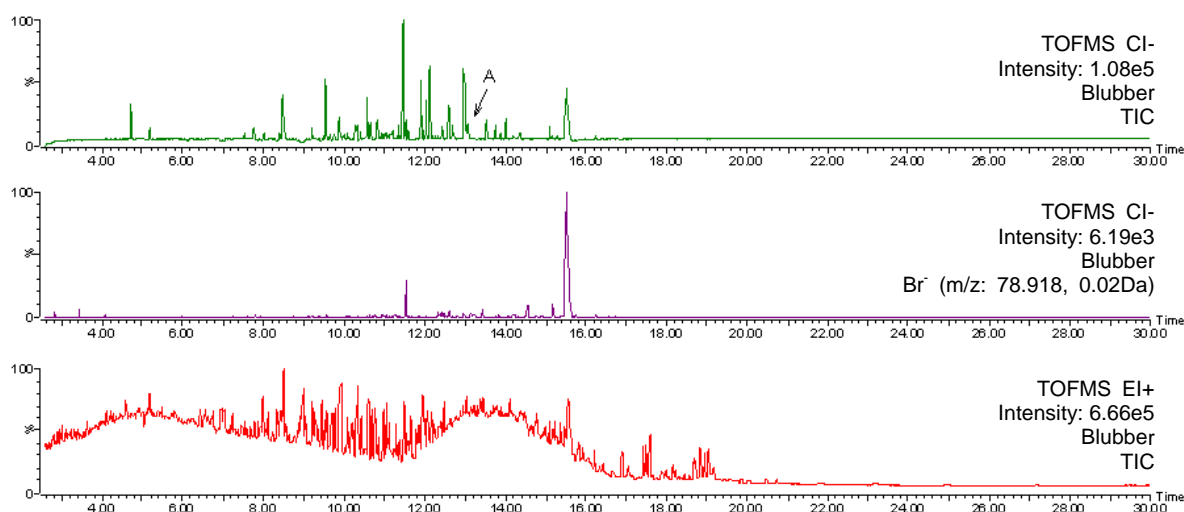


Figure 1. TIC and mass chromatogram of bromine ion obtained by GC/TOFMS with NCI measurement and TIC obtained by GC/TOFMS with EI measurement in blubber of finless porpoise.

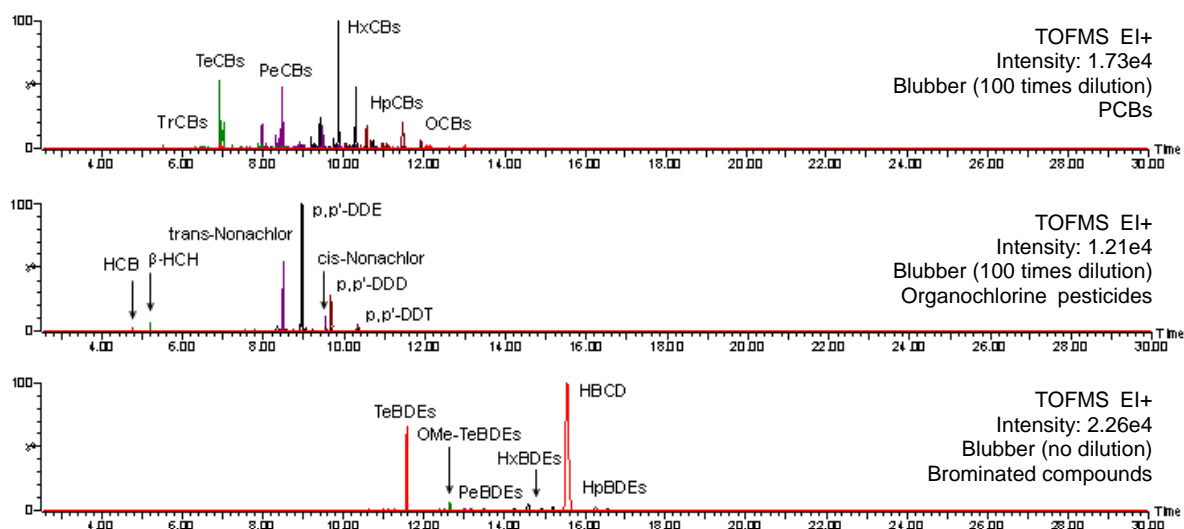


Figure 2. Mass chromatograms of PCBs, organochlorine pesticides, PBDEs and HBCD by GC/TOFMS with EI measurement. Each mass chromatograms of predominant ions detected from components were combined. Window width value of each mass chromatograms were $\Delta M = \pm 0.01$ Da.

Most of organochlorine compounds were composed of PCBs (tri- to octa-chlorinated congeners were identified, and hexachlorinated congener was predominant), HCB, β -HCH, trans- and cis-Nonachlor, p,p' -DDE, p,p' -DDD, and p,p' -DDT, and organobromine compounds were PBDEs (tetra- to hepta-brominated congeners were identified, tetrabrominated congener was predominant), OMe-TeBDEs, and HBCD (Figure 2). The contamination level of OMe-TeBDEs was less than a tenth of TeBDEs, and that of HBCD was more than that of TeBDEs.

Moreover, other organochlorine compounds were searched. Figure 3 shows the theoretical accurate mass spectrum of octachlorodiphenyl ether (OCDE) and the accurate mass spectrum of peak A (refer to Figure 1) observed by GC/TOFMS with EI measurement. The difference of accurate mass between theoretical and observed spectrum was within ± 0.01 Da. This result suggested that peak A is OCDE. Polychlorinated diphenyl ethers (PCDEs) are widely used as flame-retardants, by-products of PCP manufacture, PCB substitutes, and pesticide additive⁷. PCDEs in finless porpoise were detected as possible interference in analysis of PCDFs⁶.

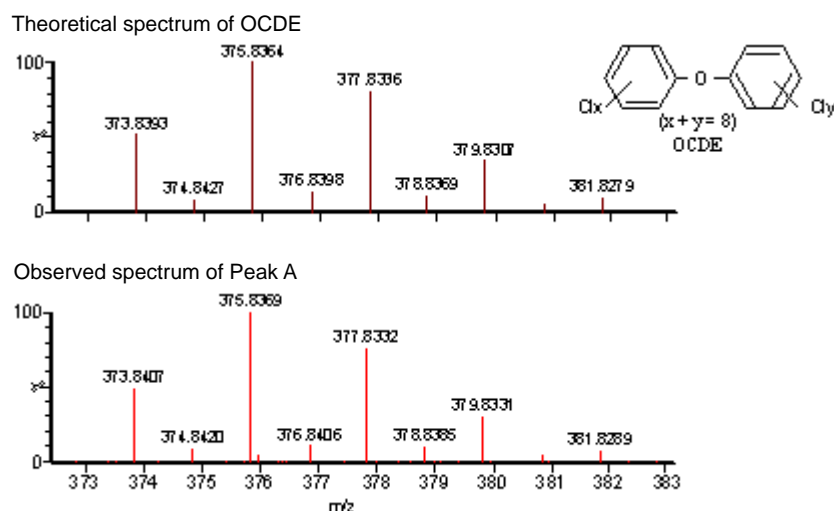


Figure 3. Theoretical accurate mass spectrum of OCDE and the accurate mass spectrum of peak A observed by GC/TOFMS EI measurement.

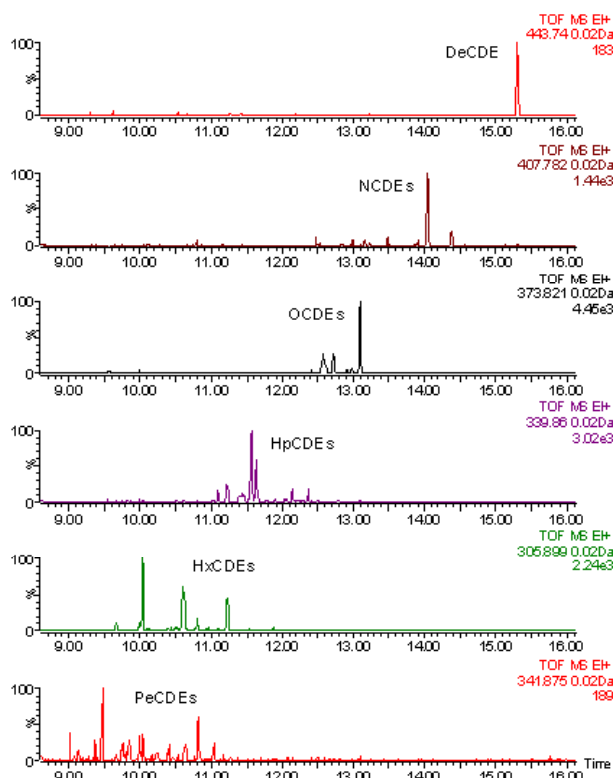


Figure 4. Mass chromatograms of PCDE congeners in blubber of finless porpoise. Window width value of each mass chromatograms were $\Delta M = \pm 0.01$ Da.

Figure 4 shows the mass chromatogram of PCDEs in blubber of finless porpoise. These mass chromatograms indicated that penta- to deca-chlorinated congeners of PCDEs had persistence and bioaccumulation potential in organisms and octachlorinated congener was predominant. The contamination level of PCDEs was less than a hundredth of PCBs, and equal or more than that of PBDEs. The accumulation pattern of congeners was different from PCBs and PBDEs.

In contamination status, PCBs were detected at the highest levels, and those of other organohalogen compounds were in the order of DDTs > CHLs > HCHs > HCB > PCDEs > HBCD > PBDEs. Contamination levels of PCBs, DDTs, and CHLs were in the order of $\mu\text{g/g}$, other organohalogen compounds were in the order of ng/g , and these results were similar to previous study for these compounds except PCDEs in blubber of finless porpoise along the Japanese coast⁸.

References

1. Watanabe, I., Sakai, S. *Environment International* 2003; 29, 665-682.
2. de Wit C.A. *Chemosphere* 2002; 46: 583-624.
3. Matsukami, H., Watanabe, K., Takemori, H., Takahashi, S., Takasuga, T., Tanabe, S. COE International Symposium 2006-Pioneering Studies of Young Scientists on Chemical Pollution and Environmental Changes, Matuyama, Japan, November, Abstract Book, 7-10.
4. Takasuga, T., Matsukami, H., Watanabe, K., Takemori, H., Takahashi, S., Tanabe, S. *Organohalogen Compounds* 2006; 68: 1178-1181.
5. Vetter, W., von der Recke, R., Herzke, D., Nygard, T. *Organohalogen Compounds* 2006; 68: 1-4.
6. Takahashi, S., Sakai, S., Watanabe, I. *Organohalogen Compounds* 2005; 67: 430-433.
7. Nimi, A.J., Metcalfe, C.D., Huestis S.Y. *Environmental Toxicology and Chemistry* 1994; 13: 1133-1138.
8. Kajiwara, N., Kamikawa, S., Ramu, K., Ueno, D., Yamada, T.K., Subramanian, A., Lam, P.K.S., Jefferson, T.A., Prudente, M., Chung, K.H., Tanabe, S. *Chemosphere* (2006) 64: 287-295.